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Color in Informatics and Media Technology (CIMET)

RGB FLUORESCENCE IMAGING BY CHANGING ILLUMINATION

Master Thesis Report

Presented by

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Abstract

Fluorescence is common phenomenon observed in many objects. In recent years, there have been an increasing number of applications of fluorescent materials and fluorescence imaging has found many application fields. Most of these applications require faster and cheaper measurement methods. In this thesis work one-monochromator and two-monochromator fluorescence measurements methods are studied and evaluated. Spectral variables and colorimetric values for the bispectrometer measurements are derived and detailed Donaldson matrix theory is provided. By analyzing RGB values of a image we show that contribution of a fluorescent component to the color of the sample is altered by illumination, thus the total reflectance and RGB values are affected. Moreover, we propose the method to estimate and recognize fluorescence from RGB values of an image.

Acknowledgments

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Joensuu, June 13th, 2012

Kristina Naumovic

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1 Introduction

In recent years, there have been an increasing number of applications of fluorescent materials and fluorescence imaging has found many application fields. Object appearance and accurate measurement of fluorescence are important in paper industry [1][2], security printing [3], biomedicine [4], and remote sensing [5][6]. Fluorescence is employed in many other applications such as art, lighting, and electronics. Some of the fluorescence applications include white LEDs used for lighting, plazma displays, fluorescent multilayer disc technology where fluorescent is used instead of reflective material for storing data, glow sticks used in military operations, banknotes. Fluorescence is applied in forensics as human body has many fluorescent elements. Most of these applications require faster and cheaper measurement methods.

Color appearance of non-fluorescent materials has been always the central point of color related computer vision algorithms, since in the standard computer vision text books and articles only regular RGB-images are considered [9]. Thus the accuracy of these color algorithms is limited. In normal color imaging systems it is difficult to preview appearance of fluorescent materials under different light sources. Traditional reflectance measurements are not sufficient as for the fluorescent opaque materials the color appearance depends on fluorescent radiation in addition to reflected radiation [12]. When non-fluorescent opaque material is irradiated part of the light is absorbed, and the rest of the light is reflected at the same wavelength. However, when fluorescent material is irradiated, part of the light is absorbed and the rest of the light is either reflected at the same wavelength or emitted at longer wavelength. As a consequence, the colorimetric values of fluorescent object may significantly differ under different illuminants.

Let us consider a fluorescent material with a narrow excitation band, i.e. only narrow wavelength band of light causes fluorescent. If the fluorescent occurs in the wavelength region of the spectrum where there is no emission of the light source, then fluorescence influence to the color of the material would be zero (see Figure 1). But if the emission of the light source is shifted by a few nm, the fluorescent component may dominate the color (see Figure 2).

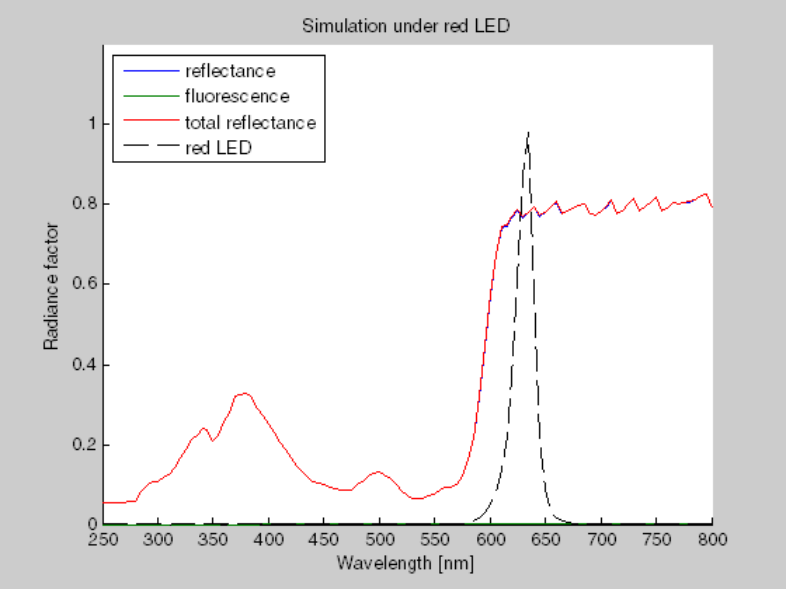

Figure 1: Fluorescent sample illuminated by red LED presented in dashed black curve, fluorescent component is zero

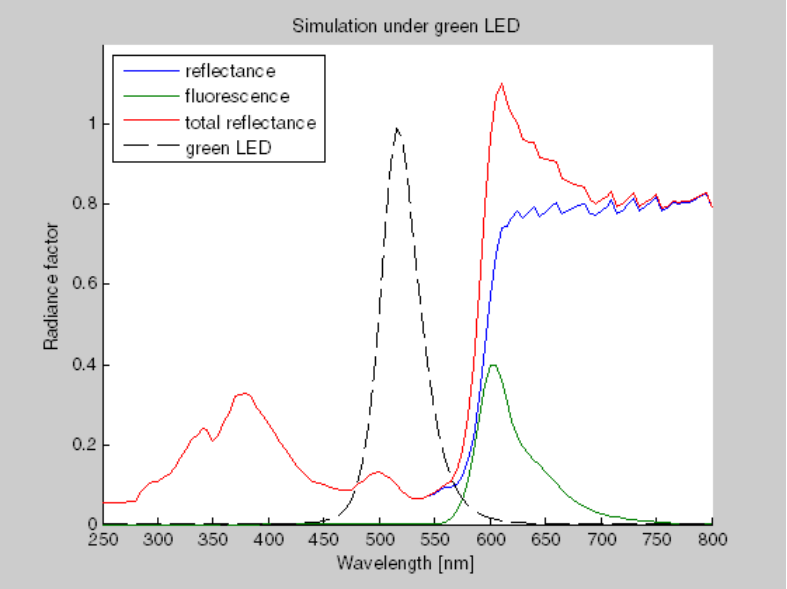

Figure 2: The same fluorescent sample as in Figure 2 illuminated by green LED which is presented in dashed black curve, fluorescent component is presented in green curve

Fluorescent sample colorimetry differs from colorimetry of a non-fluorescent sample. Conventional colorimeters or spectrophotometers are not accurate in measuring the fluorescent component of the object color because the fluorescence emission appears at the wavelengths of fluorescence absorption.

Several studies propose different fluorescence measurement methods: one-monochromator method [7], various filter methods [10][11], and two-monochromator method [14]. In one-monochromator method fluorescent sample is illuminated with polychromatic light and measurement outcome is total spectral radiance factor. Acquisition of separated fluorescent and reflected component is possible with filter methods and two-monochromator method. In filter methods sample is measured using common spectral radiance measurement techniques with spectroradiometer or spectrometer. Then one or more filters are employed in order to eliminate excitation energy and spectral radiance factor is measured again under modified conditions. Fluorescence is detected by comparing the resulting spectral curves. Two-monchromator method was proposed by R. Donaldson in 1952. [18] and is today's standard in measuring fluorescent samples. It is the most accurate method to distinguish the fluorescent characteristics of the sample. Each of these methods has advantages and drawbacks depending on the instrumentation used, and fluorescent emission wavelength and intensity.

So far, there has been no research to our knowledge that surveyed fluorescence of an object by RGB-imaging using changing illumination. In this study, several fluorescence measurement methods has been studied and evaluated. The computational methods have been used to estimate fluorescence from bispectrometer measurement and to estimate influence of fluorescence from a RGB image. The main contribution in this thesis is developed method to estimate and recognize fluorescence from RGB values of an image.

This paper has been divided into six chapters. After introducing the objectives and aims of this study while giving the brief overview of fluorescence, in chapter two theoretical dimensions of the research are laid out. Fluorescence phenomenon is described in more detail, materials that exhibit fluorescence and their applications are presented, and fluorescence in color images is reviewed. In addition, different fluorescence measurement methods are presented.

Chapter three describes the methods proposed in this research. First, bispectrometer approach is presented as the most accurate method in measuring fluorescence materials. Spectral variables and colorimetric values for the bispectrometer measurements are derived and detailed Donaldson matrix theory is

provided. Illuminants used in the measurements are presented. Furthermore, LCTF approach is proposed and light sources used in the measurements are presented.

Chapter four describes the experiment design, samples and devices used in the measurements. LCTF camera and components of the custom made bispectrometer are presented.

In chapter five obtained results with bispectrometer, and LCTF measurements are presented and analyzed. Chapter six summarizes the previous chapters, discussing the mentioned goal and the obtained results. The chapter gives a conclusion by comparing the results of samples illuminated by different light sources. In addition, some directives for the future work are given.

2 Fluorescence phenomenon

When energy levels of atoms bound in molecules are excited the result is optical radiation, this process is known as luminescence. Photoluminescence is a luminescence which is caused by ultraviolet, visible or infrared radiation. Fluorescence is photoluminescence which occurs when a material absorbs photons at some certain wavelength or group of wavelengths from ultraviolet and visible spectrum and then emits photons of a different band of wavelengths.

Fluorescence phenomenon is illustrated in Figure 3. by Jablonski diagram which describes photo physical processes in molecular system [13]. When fluorescent material absorbs light, the electrons in the material are excited from the ground state to higher vibrational energy states. The ground state and the other singlet states are divided into vibrational energy levels. S_0 presents the ground state while S_1 and S_2 present higher energy states. The vibrational energy levels are donated by 1, 2, 3, and 4. One of the absorption or excitation transitions presented in Figure 3. occurs from the ground state S_0 in its lowest vibrational level zero to the first vibrational energy level in the second excited state S_2. At this point due to loss of energy in the absence of light emission the molecule will relax to the lowest vibrational energy level of the first excited state S_1. This process is known as vibrational relaxation or internal conversion and occurs in 10^{-14} – 10^{-11} sec. Excess vibrational energy is then converted into heat which is absorbed in collision by the neighboring molecule. From S_1 the molecule often returns to some of the vibrational levels of the ground state S_0. If this relaxation is accompanied by emission of a photon the process is known as fluorescence. The fluorescence occurs in 10^{-9} – 10^{-7} sec. In the excited state S1 another phenomenon can occur if the excited molecules collides with another molecules to transfer energy, this process is known as intersystem crossing, by shifting it's spin and thus moving to lowest excited triplet state donated by T1 in Figure 3. This process can cause phosphorescence or delayed fluorescence where transition back to excited singlet state occurs. Life time of phosphorescence is between 10^{-3} – 10^{-2} sec.

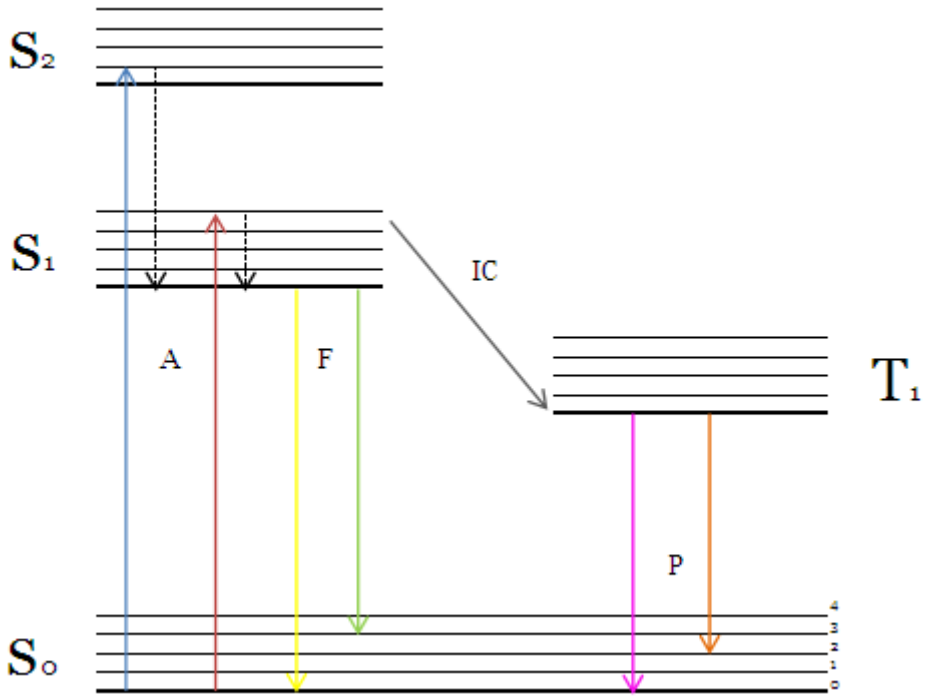

Figure 3: Jablonski diagram: A - Absorption, F - Fluorescence, IC - Intersystem crossing, P-Phosphorence, S0 – Ground state, S1 and S2 – Higher energy states, T1 – Triplet state

In fluorescence the emitted energy is less than exciting energy, thus emission wavelength is always longer than the excitation wavelength. The difference between excitation maxima and emission maxima is known as Stokes shift [15]. If emitted photon has more energy, then this difference is known as Anti-Stokes shift.

The color of the object is determined by the reflectance. When non-fluorescent material is illuminated the emission wavelength is the same as excitation wavelength and the resulted spectrum is known as reflectance, however when fluorescent material is illuminated the emission wavelength is longer than excitation wavelength, thus the resulted spectrum is the combination of reflectance and the emission spectrum.

The color of the object depends on illumination under which the object is viewed in addition to the reflectance properties of the object [33]. Color stimuli of a non-fluorescent object can be expressed as

$$\Phi(\lambda) = R(\lambda)\, S(\lambda) \tag{2.1}$$

where $R(\lambda)$ is spectral reflectance of the object and $S(\lambda)$ is spectral power distribution of the illumination under which the object is viewed.

However, when calculating color stimuli of a fluorescent object total reflectance must be taken into consideration [19]

$$R_T(\mu, \lambda) = R_R(\lambda) + R_L(\mu, \lambda) \tag{2.2}$$

where $R_T(\mu, \lambda)$ is total reflectance, $R_R(\lambda)$ is reflectance, $R_L(\mu, \lambda)$ is fluorescence, μ is excitation wavelength, and λ is emission wavelength.

Therefore, color stimuli of a fluorescent object can be expressed as

$$\Phi(\lambda) = R_T(\mu, \lambda)\, S(\lambda) \tag{2.3}$$

where $R_T(\mu, \lambda)$ is total spectral reflectance of the object and $S(\lambda)$ is spectral power distribution of the illumination under which the object is viewed.

2.1 Fluorescent materials

Fluorescent materials have been used in a variety of applications in different industries. Compared to non-fluorescent materials they have higher lightness and saturation. Commercial fluorescent pigments can be divided in three major groups: daylight fluorescent pigments, brightening agents, and inorganic fluorophors.

Daylight fluorescent pigments are materials which have excitation and emission maxima in the visible region of the spectrum; as a result they can be excited by almost any illuminant. Their application is mostly associated with their ability of intensifying the output of light at particular wavelength which makes the materials appear brighter and more colorful than ordinary materials under the same daylight illumination. They are used in the advertisement as they can attract attention; moreover daylight fluorescent colors are very effective on emergency ambulance vehicles, firefighting vehicles, etc. Very important application of these materials in combination with retro reflective materials is in traffic signs and protective garments. The advantage of daylight fluorescent pigments is in their ease of integration into textile dyes or compound into thermoplastic.

Brightening agents also known as fluorescent whitening agents have excitation maxima in near UV region, and they emit in violet and blue region of the visible spectrum. These agents are used to enhance the whiteness and improve the appearance of materials in paper, textile, and plastic industries. Laundry detergents and laundry bleaching materials contain whitening agents. Most white papers contain a fluorescent whitening agent to enhance the appearance of the product by neutralizing the yellowish cast of the material with emitted blue light.

Inorganic fluorescent materials are based on zinc sulfide or cadmium sulfide. The best known materials are based on zinc sulfide doped with copper. In the past their use has been discouraged in many applications, as most of the materials were based on zinc cadmium sulfide doped with silver which was considered as toxic. Most of these organic fluorescent materials have excitation maxima in UV region, some of them in mid-visible region and some of them even in near-IRE [16]. One of the main applications of these materials is in security printing. Use of inorganic fluorescent pigments is one of the ways to protect documents against falsification.

2.2 Fluorescent color measurement

Standard colorimeters or spectrophotometers are not accurate in measuring the fluorescent component of the object color. If the data obtained is measured by scanning the monochromatic incident light the fluorescence emission appears at the wavelengths of fluorescence absorption thus the data is useless [11].

The main methods for measuring the fluorescent sample are one-monochromator method, filter method, and two-monochromator method. One-monochromator method measures total radiance factor, however there are three ways to detect and separate the fluorescent and reflected components of the measurement instrumentally [20]. These three methods are: two-mode method, filter method, and two-monchromator method. Two-monochromator method is the most accurate method and today's standard in measuring fluorescent samples [20]. Filter methods involve measuring the sample using common spectral radiance measurement techniques with spectroradiometer or spectrometer. Then one or more filters are employed in order to eliminate excitation energy. Afterwards spectral radiance factor is measured again under modified conditions. Fluorescence is detected by comparing the resulting spectral curves. Two-mode method is based on comparing the results of two measurements [8]. One mode involves polychromatic illumination and the other monochromatic illumination. The idea is that fluorescence will show up as increased values at emission wavelengths when in polychromatic mode but not necessarily in monochromatic mode. If the fluorescence exist two spectral curves will always have different shapes [35]. The instruments which can be used in two mode method need to have changeable position of a source and detector in order to use either polychromatic illumination mode or monochromatic illumination mode.

2.2.1 One-monochromator method

In one-monochromator method the data obtained is measured by scanning the polychromatic incident light from a reference illuminant e.g. D65. The measured quantity is total spectral radiance factor without the possibility to separate reflected and fluorescent part of the spectrum [32].

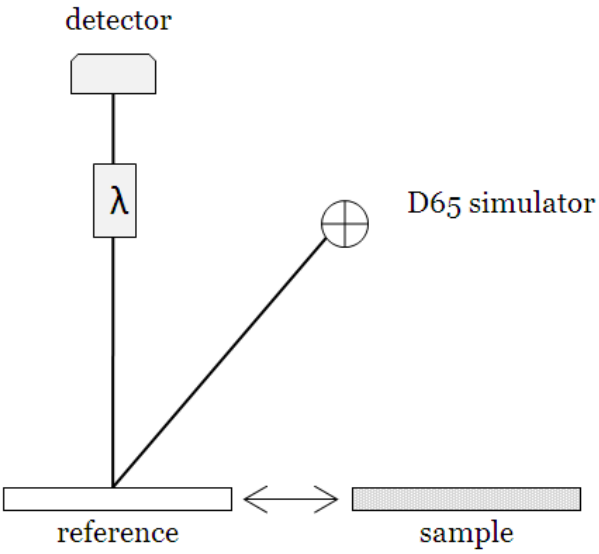

Figure 4: One monochromator method

Accurate measurement of a total radiance factor with one-monochromator method is achievable. Since fluorescent component of the total radiance factor is illuminant dependent, the total radiance factor will always be dependent on the light source used in the measurement. It cannot be used to determine the colorimetric values of the fluorescent sample under other illuminants, which is important in detecting the metamerism. For these reasons, the one-monochromator method is not used for high-accuracy traceable fluorescence measurements [37].

Sufficient calibration of one-monochromator system can only be accomplished by using fluorescent reflectance standards with optical properties similar for the samples of standard illuminant D65 in order to correct instruments readings [32].

2.2.2 Filter method

Total radiance factor is not the only spectral information of the fluorescent sample usually needed, as the amount of fluorescent radiance depends on the relative spectral distribution of the light source used. There are several methods to separate the total radiance factor into its subcomponents on a one-monochromator instrument. There are three accepted ways which involve filters [8]: fluorescence weakening method, filter reduction method, and adjustment method.

Fluorescence weakening method involves using two different bandpass filters and three measurements are compared [36]. One filter is so called fluorescence killing filter which is used to remove all fluorescence exciting wavelengths. The other filter is fluorescence weakening filter which is used to remove incident illumination about 20-40 nm shorter than first filter.

Filter reduction method uses series, usually three to five, linear long bandpass filters to estimate reflected radiance factor and no filter for total radiance factor, thus the fluorescence radiance factor can be calculated.

Adjustment method uses series of narrow bandpass filters along visible spectrum. The fluorescence presence is indicated by the difference of reflectance and total radiance curves [38]. This method requires complex calculations and uses many filters.

These filter methods along with two mode method have been developed long time ago, when the measurement devices were not advanced enough to measure fluorescent samples accurately. Since they involve using polychromatic light and filters, and are concentrated mainly to VIS region, they are not able to give accurate results. Therefore these methods are not used anymore.

For reliable measurements the two-monochromator method or almost equivalent serial filter method should be used. Serial filter method is discussed below.

The serial method was introduced by Simon [39] in 1999. It provides results that are comparable to two-monochromator method. This method uses a 10-12 sharp cut-off filters in VIS region which are selected by approximating the wavelengths where the transmission of the filter is 50% and separated by around 25 nm. Each filter reduces the level of fluorescence, revealing a short portion of the reflected radiance factor, first for white standard i.e. normalization and then for the fluorescent sample. The total radiance factor is obtained with no filter. The fluorescence is then obtained by subtraction of the total radiance factor and radiance factor.

2.2.3 Two monchromator method

The two-monochromator method or bispectrometer method is the most accurate method to define the fluorescent characteristics of fluorescent samples. It was first proposed by R. Donaldson in 1954 [18]. It is considered as the reference method for determining the colorimetric properties of fluorescent materials.

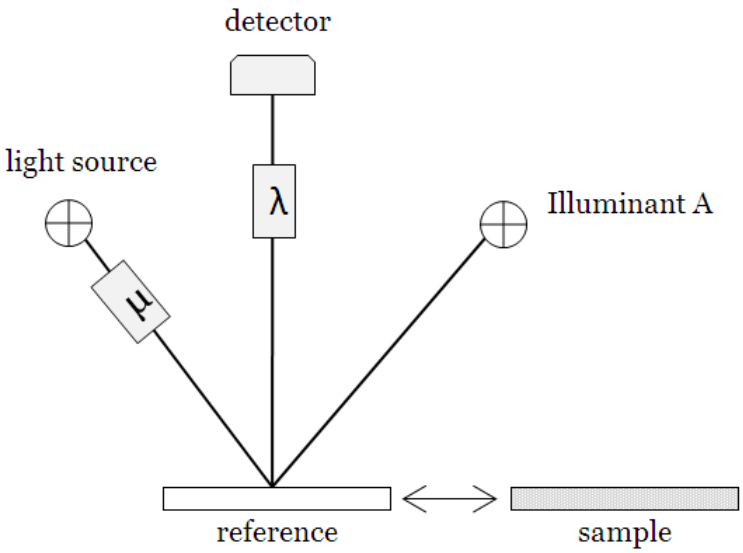

Figure 5: Two-monchromator method

The bispectrometer is used in measuring the bispectral radiance factor of the fluorescent samples. Bispectrometer is an optical instrument equipped with a source of irradiation, two monochromators, and a detection system, such that a specimen can be measured at independently-controlled irradiation and viewing wavelengths [40].

It is composed of two separated monochromators. The first monochromator, the excitation monochromator, is for separating radiation from a broad spectrum polychromatic light source into monochromatic light which then irradiates the sample. The second monochromator or the emission i.e. detection monochromator separates the radiation emitted from the sample into its spectral components. For colorimetric applications bispectrometer's emission spectrometer must be able to monitor sample radiance over the entire visible spectrum. This range is defined by CIE and it should extend at least from 380-780 nm depending of the exact parameters of the measurement. Likewise, bispectrometer's excitation spectrometer must provide illumination over the entire visible spectrum. As some of the fluorescence materials may exhibit visible emission when excited in ultraviolet region, the range of excitation spectrometer must be extended over that part of the spectrum in which the relevant illuminants provide the significant energy. The minimum recommended spectral range is 300-780 nm.

The idea of taking a bispectrometer measurement is to record a bispectral matrix of output light by scanning both the excitation and emission wavelengths. At the same time the matrix of non-fluorescent material is captured thus it is possible to compensate the effect of the measuring device and compute the Donaldson matrix [19]

- light source independent representation of fluorescent sample. Donaldson matrix is discussed in more detail in subsection 3.1.1.

Federal Institute for Materials Research and Testing, BAM in Germany and National Research Council, NRC in Canada use bispectometers that have two scanning monochromators, both of the instruments employ 45˚/0˚ measuring geometry.

NRC reference bispectrometer uses a scanning monochromator for illumination and a second monochromator with two different photomultiplier tubes in order to enable scanning range of 250 – 1050 nm with 5 nm steps. The reflected radiance factor and fluorescence spectrum are defined separately for s- and p-polarized light. The measured field of the surface of the sample is approximately 10 mm. Device uses 300 W Xenon arc lamp and has order sorting filters and polarizers for carrying out accurate measurements [14][41][42].

BAM reference two-monochromator bispectrometer was the first reference bispectrometer to be built. It uses a similar approach to NRC device. Scanning range of this device is from 300 – 800 nm with 10 nm steps. The measured field of the surface of the sample is approximately 25 mm [43].

The readings obtained directly from the device are influenced by many device specific factors. Some of them are: bandpass function of the excitation and emission monochromators, spectral power distribution of the light source, spectral responsivity of the device's detection system, spectral transmittance of the device's transfer optics, polarization errors [44].

The calibration principles generally used in all bispectrometers are similar to ones proposed in [45] by Minato et al. The standard method of compensation involves the use of internal reference detector to monitor the device's excitation beam through a beam-splitter [19]. Compensation for both temporal and spectral variations in flux incident upon the sample is possible by normalizing the readings from device's emission detection system with readings from the reference detector. In addition, emission spectrometer can be calibrated by the use of a non-fluorescent standard for a known spectral reflectance factor used in conjunction with the calibrated excitation system. This reflectance calibration can be used as bispectral radiance factor calibration for the diagonal of the bispectral matrix. Furthermore, the relative spectral calibration of the excitation spectrometer can be used to extend this calibration to off-diagonal elements of bispectral matrix.

If monitoring the device's excitation beam by reference detector is not convenient, the calibration of a bispectrometer can be realized by any of the following methods [46]:

1. Direct calibrations of both sides of the device by using the standard source and a standard detector. Instead of partial bispectral radiance factor calibration provided by the reflectance standard this method requires absolute spectral radiance and spectral responsivity calibration.

2. The excitation spectrometer is calibrated using an external detector of known relative spectral responsivity. Non-fluorescent standard for a known spectral reflectance factor is used in conjunction with this relative spectral calibration providing absolute bispectral radiance factor calibration for diagonal elements of the bispectral matrix.

3. The emission spectrometer is calibrated by using an external source of known relative spectral radiance. The calibration is transferred back to en excitation spectrometer by means of the reflectance standard.

Measurements of fluorescent samples should be performed under 45˚/0˚ or 0˚/45˚ illuminating and viewing conditions in order to minimize sample/instrument interactions [47].

45˚/0˚ condition: Illumination beams' axes are at angle of 45˚± 2˚from the normal to the surface of a sample. The angle between the direction of the viewing and the normal to the sample should not exceed 10˚. The angle between the axis and any ray of a beam should not exceed 8˚. The same conditions apply for viewing beam.

0˚/45˚condition: The sample is illuminated by the beam whose axis is at angle not exceeding 10˚ from the normal to the surface of a sample. The sample is viewed at angle of 45˚± 2˚from the normal. The angle between the axis and any ray of a beam should not exceed 8˚.The same conditions apply for viewing beam [47] [48].

If sphere instruments and d/0˚conditions are used in the measurements several errors will occur. Specular component excluded and the smallest available aperture should be used to perform the measurements in order to reduced the errors caused by self illumination from a sample. References [49][50] recommend not to use instruments equipped with integrating sphere in measuring fluorescent samples.

2.3 Fluorescence in color images

In many areas of computer science the accurate color reproduction is very important, thus the color of illuminated objects must be presented accurately. The color appearance of object under one illuminant is used in color imaging to identify objects, as well to predict their appearance under other illumination conditions. The perceived color of an object is determined by the spectral composition of the combination of the illuminant and the object's reflectance.

In computer graphics the rendering techniques and rendering platforms parameterize the perceived color by RGB values. That is, the reflectance and the illuminants are approximated by red, green, and blue color channels. To obtain the observed spectrum of a pure fluorescent surface, the overall contribution from its illuminant, excitation and emission spectrum must be considered.

When dealing with fluorescent objects or surfaces traditional image reproduction algorithms have significant limitations, as mentioned in the previous chapter, because they share a common assumption that none of the objects in the scene exhibit fluorescence.

Glassner was first to include fluorescence and phosphorescence in rendering algorithms by extending rendering equation presented by Kajiya in his 1884 paper [28]. Johnson and Fairchild provided brief theory of fluorescence and extended spectral rendering algorithms to take fluorescence into account [29].

In color related computer vision algorithms the same issue is addressed. In [27] different color constancy algorithms with a large set of test images including images of fluorescent objects were evaluated and it is showed that algorithms assumed fluorescence as normal reflective component. They proposed methods to improve color constancy algorithms so they can deal with spectral data of several fluorescent materials.

For example, if we want to predict how the object will look like under the red light, the image can be relighted by using the traditional relighting algorithms, however the fluorescent colors in the image will not be reproduced accurately. This proves that reflective and fluorescent components interact with illuminants differently. To achieve accurate relighting, it is required to separate the components and process each component separately.

Lee and Chen [17] proposed the method to estimate the reflected and fluorescent spectral radiance factors based on spectrophotometric data without using a monochromator. Both of the factors are approximated by truncated Fourier series. Furthermore, with special weighted least square algorithm they are able to provide good color prediction of the fluorescent samples under different light sources.

In the area of computer vision several methods for separating components of the image are proposed [30][31]. Some of the algorithms separate non-correlated components of the image, and some of them separate specular reflection from diffuse reflection.

Zhang and Sato studied the possibilities to separate reflective and fluorescent components of an image and proposed the method for separation using the images captured under two illuminants [24]. They showed that color appearance of the object

containing both fluorescent and reflective component can be presented as a linear combination of these two components. By using linear contribution model they showed that reflective and fluorescent component of the image taken under two different illuminants can be effectively recovered using independent component analysis.

3 Methods

In this study we performed color measurements of various fluorescent samples with bispectrometer and LCTF camera. In this chapter we introduce the methods used for fluorescence measurements and for recognizing fluorescence from RGB values of an image.

3.1 Biospectrometer

In conducting the color measurements if the fluorescent sample is measured by scanning the monochromatic incident light the data obtained is inadequate as the fluorescence emission in data appears at the wavelengths of fluorescence absorption. If the sample is measured using white polychromatic light by scanning the detection wavelength the measured data is accurate only for the used light source and cannot be applied in simulation under any other light sources. The most accurate method to distinguish the fluorescent characteristics of the sample was proposed by R. Donaldson [18] - two monochromator method which is today's standard in measuring fluorescent samples [20]. In this method wavelengths of both incident and detected light are scanned and matrix of the output light is gathered. At the same time the matrix of the non-fluorescent reference material is captured in order to calculate the illuminant independent representation of the fluorescent sample, known as the Donaldson matrix. Once obtained the Donaldson matrix can be used to calculate the color values of the sample for any desired light source or observer.

The bispectrometer was used to record the total spectral radiance factor, spectral reflected radiance factor which is light source independent, and the spectral fluorescent factor which is light source dependent. The data obtained with bispectrometer for one sample is fluorescence matrix F(μ,λ) where each row donates the emission spectrum at corresponding excitation wavelengths. λ and μ present the emission and excitation wavelengths respectively. Donaldson matrix is obtained by dividing each row of the fluorescence matrix by corresponding intensity of the light source and peak half-width as presented in the following equation [19]

$$D(\mu,i)=\frac{F(\mu,i)}{E_{ill}(\lambda)E_{ill,half}(\lambda)},\ \forall i=\lambda \qquad (3.1)$$

Example of Donaldson matrix is presented in Figure 6.

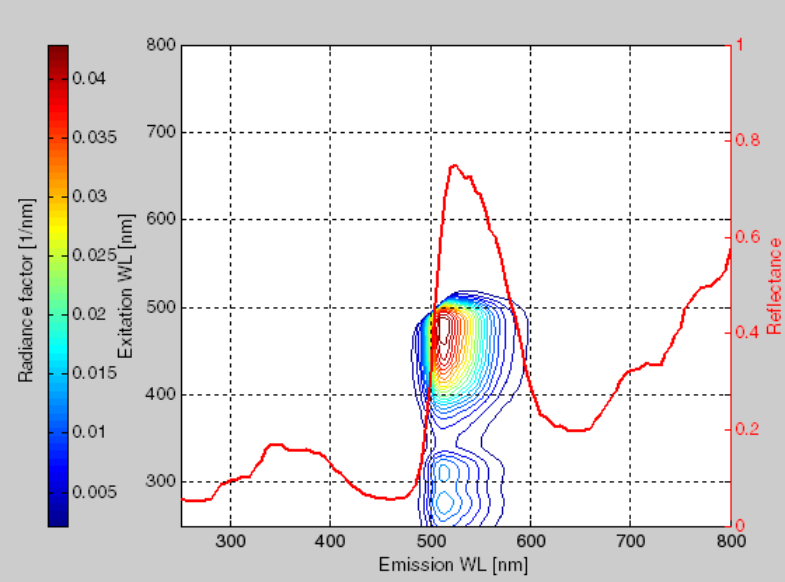

Figure 6: Green fluorescent sample: excitation spectrum (250-510 nm) and emission spectrum (490-600 nm)

The reflectance radiance factor of the sample $\beta_R(\lambda)$ is obtained from the diagonal of the Donaldson matrix

$$\beta_R(\lambda) = D\,(\mu = \lambda, \lambda) \tag{3.2}$$

When performing the simulation with chosen light source the fluorescence part of the Donaldson matrix is calculated by summing up each emission spectrum of the weighted Donaldson matrix.

The fluorescence radiance factor $\beta_L(\lambda)$ is obtained by dividing obtained fluorescence spectrum by the spectral intensity of the light source. Total radiance factor $\beta_T(\lambda)$ is obtained as a sum of radiance factor and fluorescence factor

$$\beta_T(\lambda) = \beta_R(\lambda) + \beta_L(\lambda) \tag{3.3}$$

The example result of green fluorescent paper simulated under A illuminant is shown below in Figure 7. Red curve presents total radiance factor, blue curve presents simulated reflected radiance factor, and green curve presents fluorescent radiance factor.

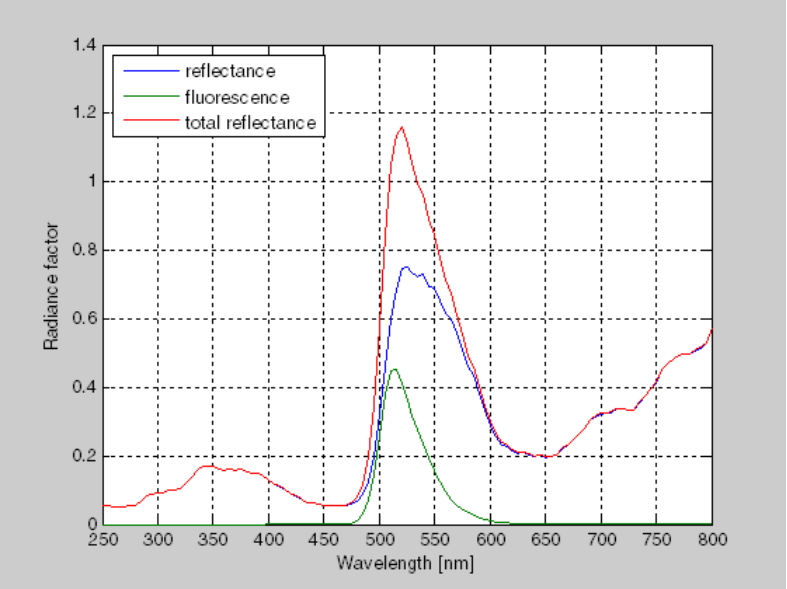

Figure 7: Simulated reflected radiance factor, fluorescent and total radiance factor under A illuminant

CIE illuminants represent light sources that are encountered in everyday life such as incandescent lamp, different periods of daylight, and fluorescent lamps. CIE standard illuminants are used in colorimetry in order to calculate tristimulus values of object color under the specified illumination conditions.

When performing the simulation following standard CIE illuminants are used: A, D50, D65, F2, and F11.

Illuminant A is CIE standard illuminant which represents incandescent light source found at home. A illuminant correlated color temperature is 2856K [21][34]. Relative spectral power distribution of A illuminant is shown below in Figure 8. This standard was introduced in 1931. In practice a gas-filled coiled tungsten filament lamp can be used for creating a light source which approximates A illuminant.

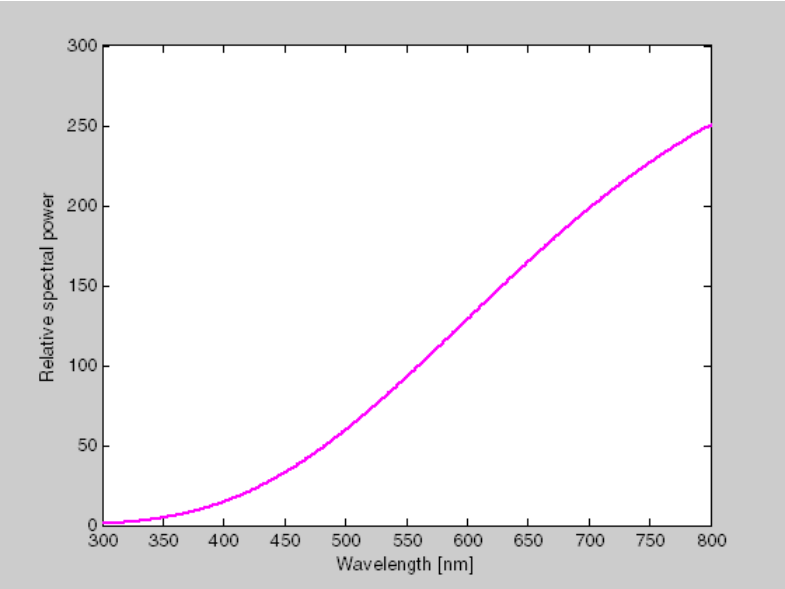

Figure 8: Relative spectral power distribution of CIE standard illuminant A

Illuminant D65 is CIE standard illuminant which represents average daylight with a correlated color temperature of approximately 6500K [21]. It was recommended in 1964 for all calculations that involve daylight, unless there are specific reasons for using other illuminant. D50 is also one of the CIE standard illuminants which represent warm daylight at sunrise or sunset with correlated color temperature of 5003K. D65 illuminant is generally used in textile, plastic and paint industries while D50 is mostly used in photography and graphics art. CIE has not yet recommended any standard light source to realize illuminant D65 and D50. However, in [23] two indoor daylight illuminants are recommended for general colorimetric and graphic art use.

Relative spectral power distributions of D65 and D50 illuminants are shown in Figure 9.

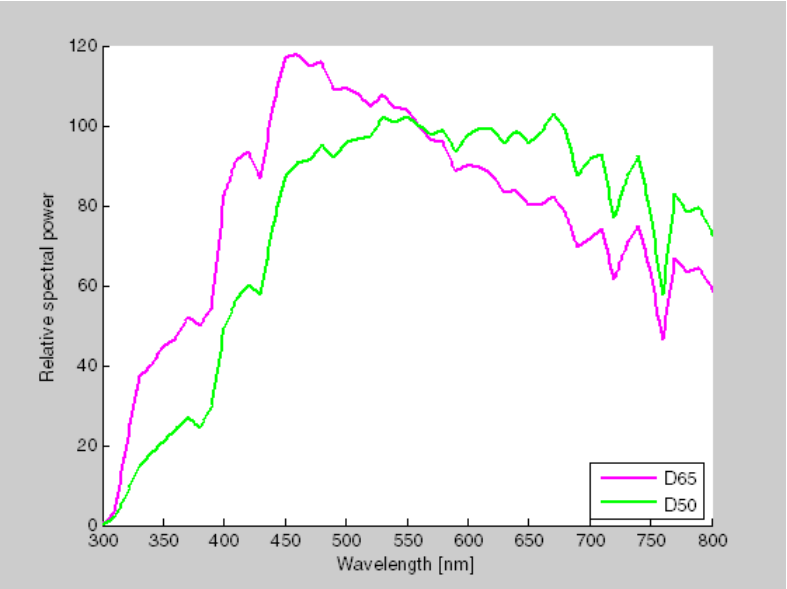

Figure 9:Relative spectral power distributions of CIE standard illuminants D65 presented in pink curve and D50 presented in green curve

CIE defined 12 types of fluorescent illuminants which represent different types of fluorescent light sources. According to emission spectra of the light source they represent they are divided into three groups. First group include standard fluorescent lamps: F1, F2, F3, F4, F5, and F6. Second group include broadband fluorescent lamps: F7, F8, and F9. Finally third group consists of three-narrow-band fluorescent lamps: F10, F11, and F12. F2, F7, and F11 illuminants are employed in colorimetry [22].

Illuminant F2 is CIE fluorescent illuminant which is in the most common use and is most similar to typical fluorescent lights which are found in offices, stores, and homes. It represents standard cool white fluorescent lamp with color temperature of 4230K. Relative spectral power distribution of F2 illuminant is shown in Figure 10.

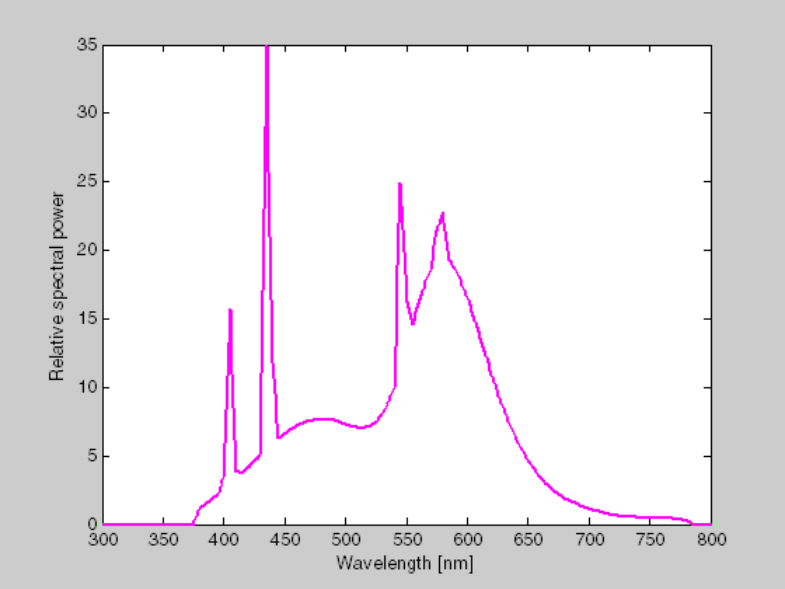

Figure 10:Relative spectral power distribution of CIE standard illuminant F2

CIE Illuminant F11 represents three-narrow-band fluorescent lamp with correlated color temperature of 4000K. F11 spectrum consists of three narrow band emissions in blue, green and red regions. When these emissions are combined together they produce highly efficient white light. Relative spectral power distribution of F11 illuminant is shown in Figure 11.

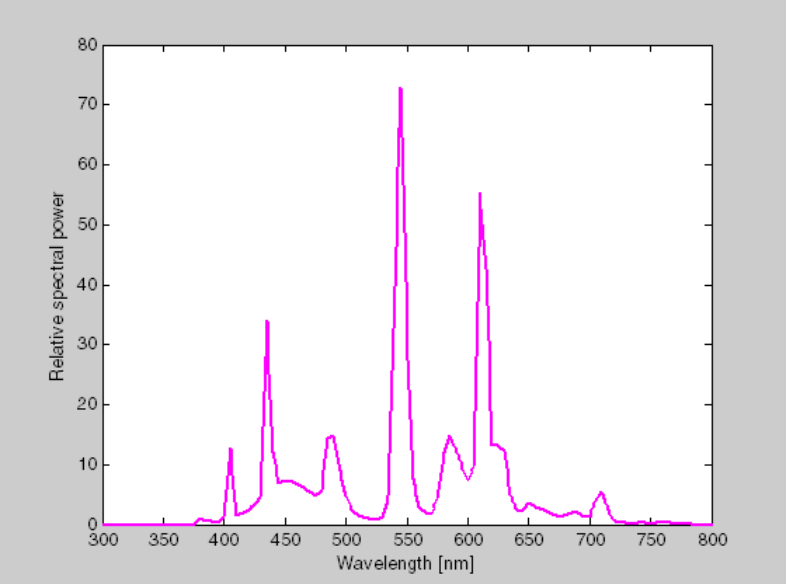

Figure 11: Relative spectral power distribution of CIE standard illuminant F11

In addition, we used white, blue, green, and red LED in the simulations. Spectral power distributions of these LEDs are shown in Figure 12.

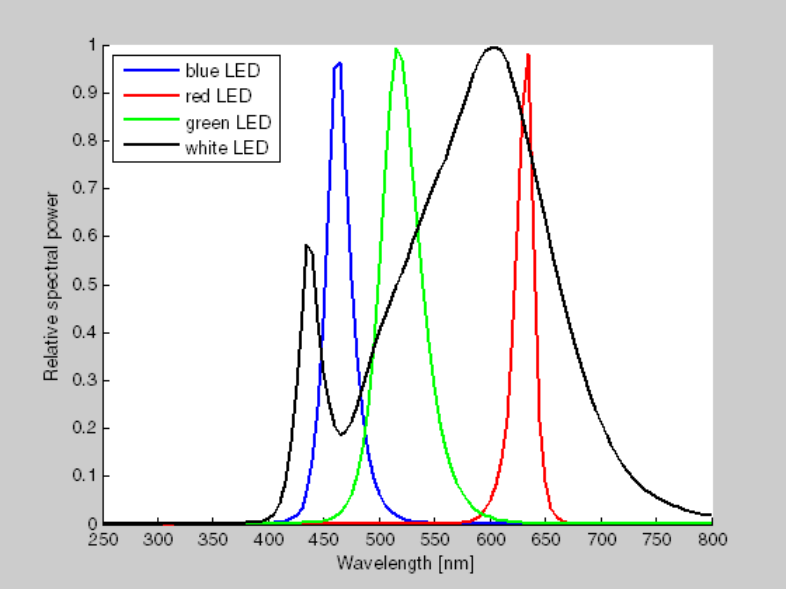

Figure 12: Spectral power distributions of LEDs

With obtained data the XYZ tristimulus values and RGB coordinates were calculated.

CIE 1931 standard colorimetric observer or CIE 2˚observer is based on color matching functions $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$ which represent color matching results of the average human population. CIE recommends that colorimetric specifications of color stimuli to be based on these color matching functions whenever the color stimuli is between 1˚ and 4˚ of visual angle. 2˚ visual field represents a diameter of approximately 17 mm at viewing distance of 0,5 m. These color-matching functions are given in the standard as values from 360 nm to 830 nm at 1 nm intervals [21]. Color matching functions for CIE 1931 standard colorimetric observer are shown in Figure 13.

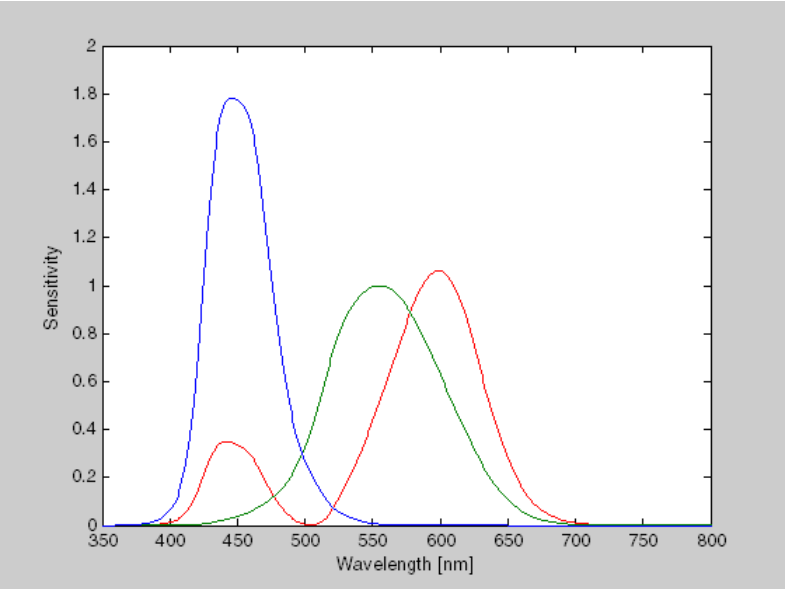

Figure 13: CIE Color matching functions for 1931 standard colorimetric observer, $\bar{x}(\lambda)$ is presented in red curve, $\bar{y}(\lambda)$ is presented in green, and $\bar{z}(\lambda)$ is presented in blue curve

When the color stimuli is more than 4˚ of visual angle CIE recommends 1964 standard colorimetric observer or CIE 10˚observer and the colorimetric specifications of color to be based on color matching functions $\bar{x}_{10}(\lambda)$, $\bar{y}_{10}(\lambda)$, $\bar{z}_{10}(\lambda)$. 10° visual field represents a diameter of approximately 90 mm and viewing distance is 0,5 m. As with 1931 standard observer, these color-matching functions are given in the standard as values from 360 nm to 830 nm at 1 nm intervals [21].

In this thesis work we used CIE 1931 standard colorimetric observer and values from 380 nm – 780 nm with 5 nm interval as it is consistent with the standard and sufficient with most applications.

CIE recommendations [21] for calculation of tristimulus values is to multiply the values of the color stimulus function $\Phi(\lambda)$ at each wavelength by that of each of

color matching functions, and to integrate each set of products over the wavelength range which corresponds to entire visible spectrum, 360 nm – 830 nm.

$$X = k \int_{\lambda} \Phi(\lambda)\, \bar{x}(\lambda)\, \Delta\lambda$$

$$Y = k \int_{\lambda} \Phi(\lambda)\, \bar{y}(\lambda)\, \Delta\lambda \qquad (3.4)$$

$$Z = k \int_{\lambda} \Phi(\lambda)\, \bar{z}(\lambda)\, \Delta\lambda$$

Where X, Y, and Z are tristimulus values, $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$ are color matching functions and k is normalizing constant which is defined below.

$$k = \frac{100}{\sum_{\lambda} S(\lambda)\ \bar{y}(\lambda)\, \Delta\lambda} \qquad (3.5)$$

For the reflecting object the color stimulus $\Phi(\lambda)$ is calculated by multiplying the spectral reflectance of an object with the spectral power distribution of the light source.

$$\Phi(\lambda) = R(\lambda)\, S(\lambda) \qquad (3.6)$$

In most cases relative reflectance factor which is normalized by dividing with the reflectance of perfect reflecting diffuser is used in the calculations. Then tristimulus values are calculated by multiplying normalized reflectance and spectral power distribution of light source by chosen standard observers, and finally integrated as presented below

$$X = k \sum_{\lambda} R(\lambda) S(\lambda)\ \bar{x}(\lambda)\, \Delta\lambda$$

$$Y = k \sum_{\lambda} R(\lambda) S(\lambda)\ \bar{y}(\lambda)\, \Delta\lambda \qquad (3.7)$$

$$Z = k \sum_{\lambda} R(\lambda) S(\lambda)\ \bar{z}(\lambda)\, \Delta\lambda$$

$$k = \frac{100}{\sum_{\lambda} S(\lambda)\ \bar{y}(\lambda)\, \Delta\lambda} \qquad (3.8)$$

Normalizing constant k is introduced in order to assure that calculations for a perfect reflecting diffuser will always give the result of luminance factor of Y equal to 100. Luminance factor is the ratio of the luminance of an object to that of a perfect reflecting diffuser, when viewed under the same lighting conditions and geometry conditions [22].

When calculating tristimulus values of fluorescent sample we need to take into consideration total reflectance of the object $R_T(\mu, \lambda)$ which is the sum of radiance factor and fluorescent factor

$$\Phi(\lambda) = R_T(\mu, \lambda)\, S(\lambda) \tag{3.9}$$

thus the equation (3.7) is modified

$$\begin{aligned} X &= k \sum_\lambda R_T(\mu,\lambda)\, S(\lambda)\ \bar{x}(\lambda)\, \Delta\lambda \\ Y &= k \sum_\lambda R_T(\mu,\lambda)\, S(\lambda)\ \bar{y}(\lambda)\, \Delta\lambda \\ Z &= k \sum_\lambda R_T(\mu,\lambda)\, S(\lambda)\ \bar{z}(\lambda)\, \Delta\lambda \end{aligned} \tag{3.10}$$

where

$$k = \frac{100}{\sum_\lambda S(\lambda)\ \bar{y}(\lambda)\, \Delta\lambda} \tag{3.11}$$

By having the possibility to obtain separate total reflectance factor, fluorescent factor and reflectance factor we were able to calculate XYZ values for each of these factors.

After obtaining XYZ tristimulus values we calculated RGB values of a image using sRGB model. Negative RGB values were replaced with zero and values greater than one were replaced with 1. Finally, the values were gamma corrected.

sRGB color space is standard color space for color management and Internet. This color space is established on aggregating the existent color strategies by enabling the method which utilizes device independent color definition for handling color in device drivers, operating systems, and Internet. It is based on a calibrated RGB color space which was already suitable for digital cameras, television, CRT monitors, scanners, and printers.

The transformation from XYZ color space to sRGB color space is given by

$$\begin{bmatrix} sR \\ sG \\ sB \end{bmatrix} = \begin{bmatrix} 3.2410 & -1.5374 & -0.4986 \\ -0.9692 & 1.8760 & 0.0416 \\ 0.0556 & -0.2040 & 1.0570 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \end{bmatrix} \tag{3.12}$$

Primaries of sRGB color space have a more limited gamut then imaginary primaries of XYZ color space thus it is possible that transformation in equation (3.12) will result in values that are negative or above 1. If this is the case then sRGB values need to be mapped to 0-1 range because the color is outside the sRGB gamut. Simple clipping is the most used method, however there are many other more complicated algorithms available. With gamut clipping only the colors that are outside the sRGB

gamut are affected. The colors that lay inside sRGB gamut are unaffected. As already mentioned negative RGB values were replaced with zero and values greater than one were replaced with 1.

Finally, sRGB values were gamma corrected, in another words transformed to non-linear values.

$$\begin{aligned}
&\text{if } R, G, B \leq 0.00304 \\
&R' = 12.92 \times R \\
&G' = 12.92 \times G \\
&B' = 12.92 \times B \\
&\text{else if } R, G, B > 0.00304 \\
&R' = 1.055 \times R^{(1/2.4)} - 0.055 \\
&G' = 1.055 \times G^{(1/2.4)} - 0.055 \\
&B' = 1.055 \times B^{(1/2.4)} - 0.055
\end{aligned} \tag{3.13}$$

After obtaining RGB values of an image we compare RGB values derived from the same image taken under different light sources and try to recognize weather there is a fluorescence contribution to the color of a sample.

3.2 LCTF

LCTF camera was used to take images of the samples in the Gretag-Macbetch Spectralight III light boot using the available light sources of the light boot. In addition to fluorescent samples a non-fluorescent sample Macbeth color checker was used as a reference in color measurements. The simulator includes UV-region light source, light source A, D65, TL84, cool white and horizon light source. The measurements were taken under mentioned light sources. In addition, UV light is added to each of the light sources and we performed measurements.

The measured quantity obtained with LCTF is total spectral radiance factor without the possibility to separate reflected and fluorescent part of the spectrum.

XYZ tristimulus values of the fluorescent material can be calculated for the spectral power distribution of the illuminant, S(λ) and color matching functions. $\bar{x}(\lambda)$, $\bar{y}(\lambda)$, $\bar{z}(\lambda)$ in the usual way from total spectral radiance factor $R_T(\mu, \lambda)$

$$\begin{aligned}
X &= k \sum_{\lambda} R_T(\mu, \lambda)\, S(\lambda)\, \bar{x}(\lambda)\, \Delta\lambda \\
Y &= k \sum_{\lambda} R_T(\mu, \lambda)\, S(\lambda)\, \bar{y}(\lambda)\, \Delta\lambda
\end{aligned} \tag{3.14}$$

$$Z = k \sum_{\lambda} R_T(\mu, \lambda)\, S(\lambda)\, \bar{z}(\lambda)\, \Delta\lambda$$

Where k is normalizing constant

$$k = \frac{100}{\sum_{\lambda} S(\lambda)\, \bar{y}(\lambda)\, \Delta\lambda} \tag{3.15}$$

With obtained XYZ tristimulus values RGB values can be calculated same as explained in subsection 3.1 in equations (3.12) and (3.13).

LCTF approach is based on idea of choosing two samples which have similar color and compare their RGB values derived from images taken under the same illuminant. By this comparison we try to recognize if there is a fluorescence contribution to the color of a sample.

4 Experiments

4.1 Samples

Samples used in this study were mostly paper, fabric, and plastic fluorescent materials. 27 fluorescent samples shown in Figure 14. were measured by bispectrometer and LCTF camera.

Figure 14: Samples used in study

4.2 Bispectrometer

As mentioned earlier bispectrometer is an optical instrument equipped with a source of irradiation, two monochromators, and a detection system, such that a specimen can be measured at independently-controlled irradiation and viewing wavelengths.

Bispectrometer used in this thesis study is custom made spectral measuring device assembled on the optical table. The set up is composed of: Xenon Arc lamp: OSRAM XBO 450W, Bentham DTMc300 Multiple-Grating Double Monochromator, Glen-Taylor polarizer, Hamamatsu Spectrometer c10027-01, traveling stage capable of holding up to four samples (see figure 15 and 16). White reference and three samples are measured simultaneously by using multiple linear polarization angles. The measurements spectral data was taken in the wavelength range of 250nm-800nm with

5 nm resolution. The measuring geometry was standard 45˚/0˚. A custom programmed software is controlling data acquisition and components of a device.

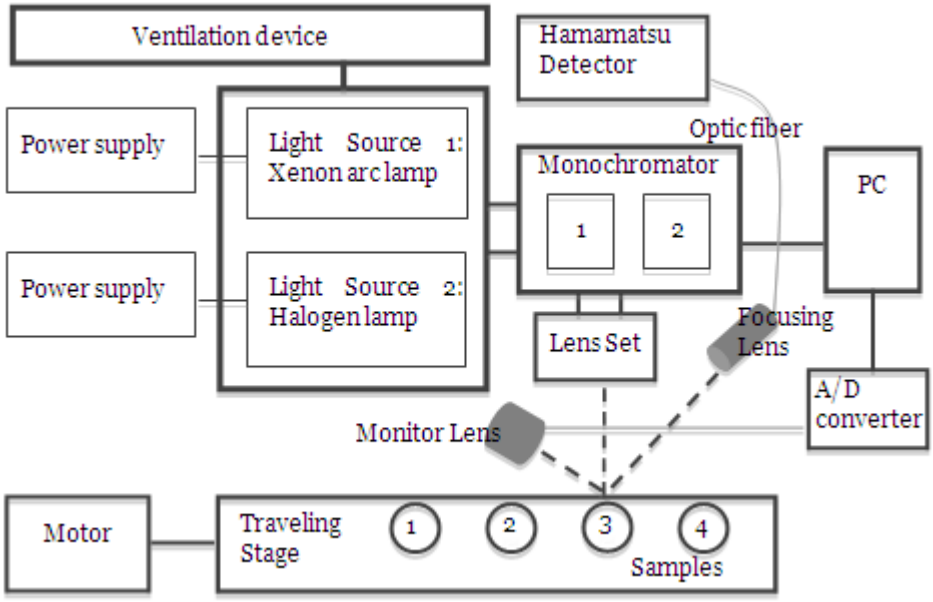

Figure 15: Bispectrometer schematic diagram

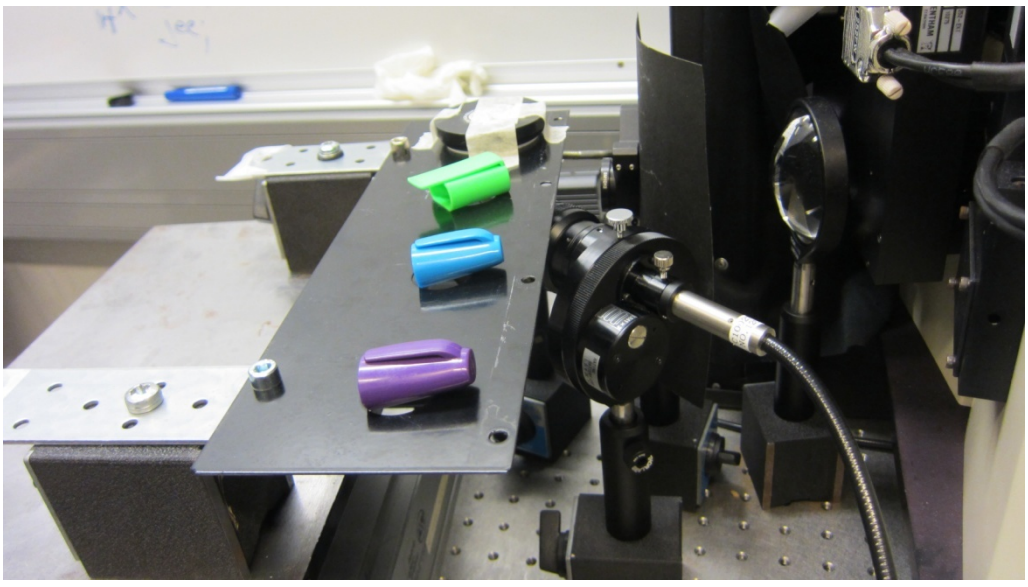

Figure 16: Bispectrometer experimental set up

Light source used in the experiment is Xenon Arc lamp: OSRAM XBO 450W, Housing: Oriel M-60923. XBO lamps are double-ended short-arc discharge lamps in which the discharge arc burns between the two electrodes in an atmosphere of pure xenon gas. Following are few of the advantages in using this lamp: very high luminance (point light source), daylight color temperature of approximately 6 000 K, high color rendering index (Ra > 96), continual color quality, irrespective of lamp type and lamp wattage.

Monochromator used in the experiment is Bentham DTMc300 Multiple-Grating Double Monochromator. Monochromator specification is presented in the Table 1.

Specification	
Configuration	Czerny-Turner
Focal length	600mm
Slits	10µm to 10mm variable, motorized
Slit height	20mm
Number of gratings	3
Grating size	68 x 84mm
Aperture ratio	f/4.1 (at all grating angles)
Resolution - additive	0.05nm at reduced slit height, 0.15nm with full slit height of 20mm, both measured with 1200g/mm grating
Resolution - dispersive	0.1nm at reduced slit height, 0.3nm with full slit height of 20mm, both measured with 1200g/mm grating
Dispersion - additive	1.35nm/mm (1200g/mm)
Dispersion - subtractive	2.7nm/mm
Mechanical resolution of grating drive	0.00072 degrees per motor step
Wavelength acquisition speed	1000nm/sec
Wavelength accuracy	+/- 0.2nm over full range of 1200g/mm grating
Wavelength responsivity	+/-0.5nm (1200g/mm)

Table 1: Bentham DTMc300 Multiple-Grating Double Monochromator specification

Traveling stage is connected with motor and can be controlled by PC. The upper part is a metal stage, which has four holes for putting samples. The bottom part moves, then horizontally shifts the upper metal stage. Four samples can be sent to the

detecting position sequentially. In this experiment white reference was measured with three different samples at the same time.

Detector is Hamamatsu spectrometer c10027-01. It is a compact spectral measurement device that combines a spectrometer and optical detector into one unit. An optic fiber is used. The wavelength axis and spectral response characteristics are already calibrated. This model uses a thermoelectrically cooled, back-thinned CCD linear image sensor that with higher sensitivity and lower noise.

Monitor detector is a set of optic lens that receives the reflected light from the sample directly. The signal from monitor detector will be transmitted to A/D convertor then transferred to the computer.

The bispectrometer was calibrated before the measurement took place.

First step in actual measurement acquisition is saturation check up. Before the real measurement starts, a test measurement must be carried out for the whole range of wavelengths. We can check whether our exposure time and amplitude settings are suitable. The input of saturation check Matlab function is the output of the test measurement. It plots a curve of the detector response. Figure 17 shows the green curve which is the response limit of the detector. The blue-red curve is the reflectance of white reference, which should not be too close to the saturation level.

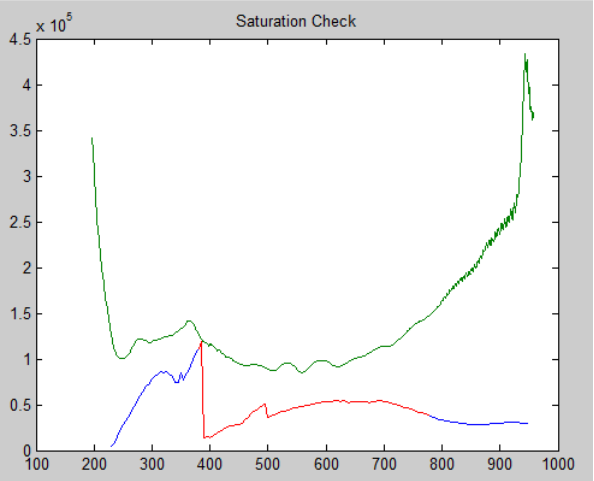

Figure 17: Saturation check graph

The goal of adjusting exposure time and amplitude is to maximize the response of the detection. Through checking the response curve and saturation curve, we can evaluate the suitable exposure.

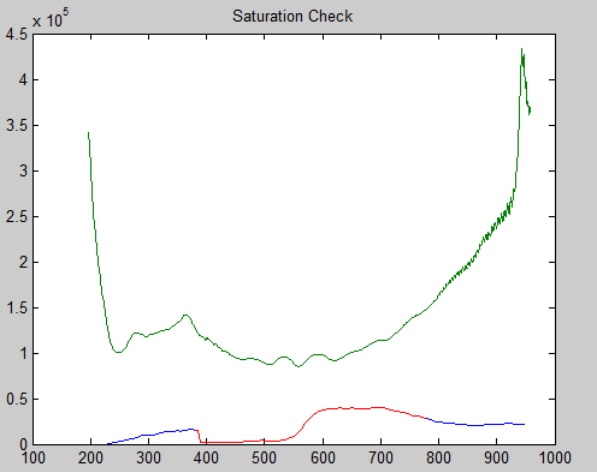

Figure 18: Fluorescent sample saturation check

Figure 18 shows the green curve which is the response limit of the detector. and blue-red curve is the reflectance of the fluorescent sample which is not close to the saturation level.

4.3 LCTF

LCTF camera used in this experiment (see Figure 19) is Nuance which operates in wavelength region 420 – 720 nm with resolution of 10 nm. The measuring geometry used is 45˚/0˚. It is composed of a lens, liquid crystal tunable filter - LCTF which is electronically controlled, optical coupler, and CCD. The camera was calibrated before the measurements took place.

Figure 19: Experimental setup, LCTF camera

Gretag-Macbetch Spectralight III light boot includes five selectable light sources: simulated daylight (D65, D50), horizon light which represents early morning sunrise or afternoon sunset, illuminant A which represents incandescent home lighting, cool white fluorescent (CWF), custom fluorescent (TL84), and ultraviolet light which can be used in conjunction with another light source. D65 distribution is relatively good and remains stable four hours after it was switched on.

5 Results and analysis

In this chapter the results of the experiments described in section 4 are presented. Simulated results are obtained from bispectrometer measurements and actual image results are obtained from LCTF camera.

5.1 Bispectrometer

Bispectrometer was used to record the total spectral radiance factor, spectral reflected radiance factor, and the spectral fluorescent factor. In Figures 20-22 reflected radiance factor is shown with red curve, the contour in the graph shows the fluorescence radiance factor with corresponding excitation and emission wavelengths which are shown on the left and at the bottom of the figure respectively. The color bar on the left side of the graph represents the bispectral radiance factor of the fluorescence.

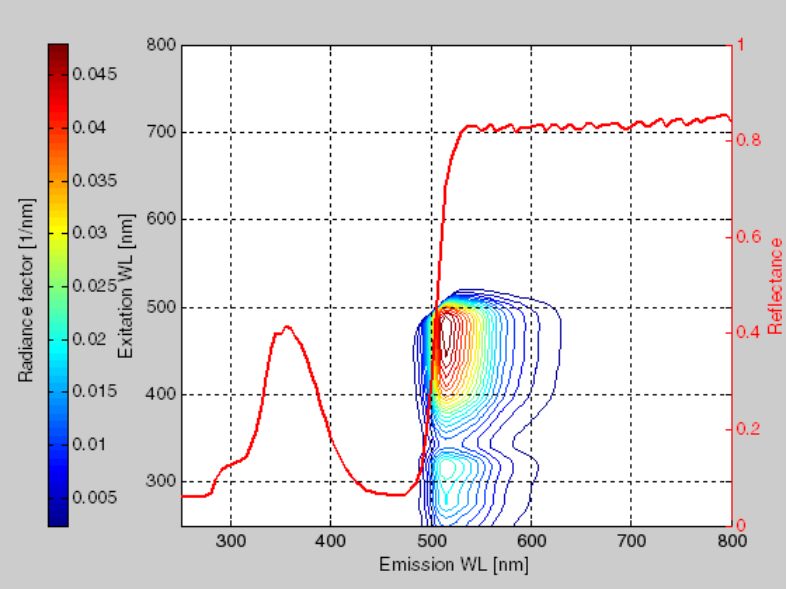

Figure 20: Fluorescent yellow sample

Figure 20 shows that excitation and emission wavelength bands of fluorescent yellow sample are in the range of approximately 250-510 nm and 490-600 nm respectively, which means when this sample is illuminated with the light source that irradiates in the region of 250-510 nm, the fluorescence of the sample is detected in the region of 490-600 nm.

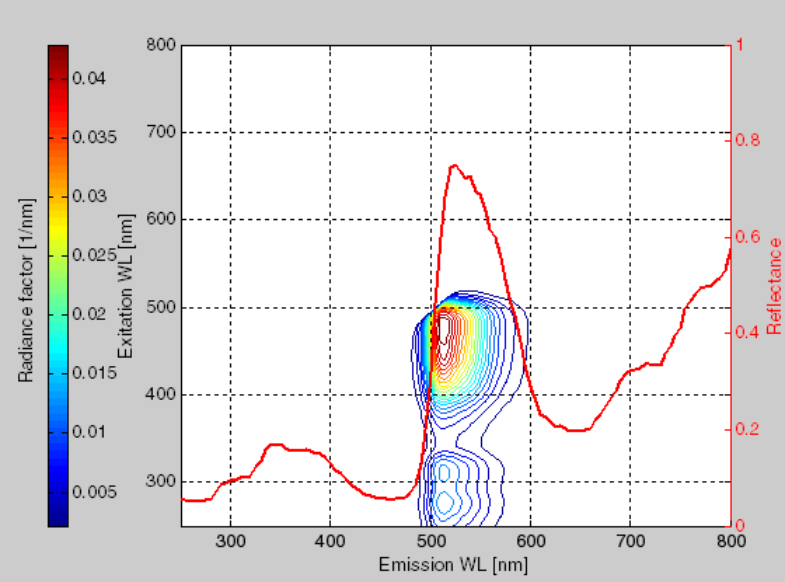

Figure 21: Fluorescent green sample

Figure 21 shows that excitation and emission wavelength bands of fluorescent green sample are in the range of approximately 250-510 nm and 490-590 nm respectively.

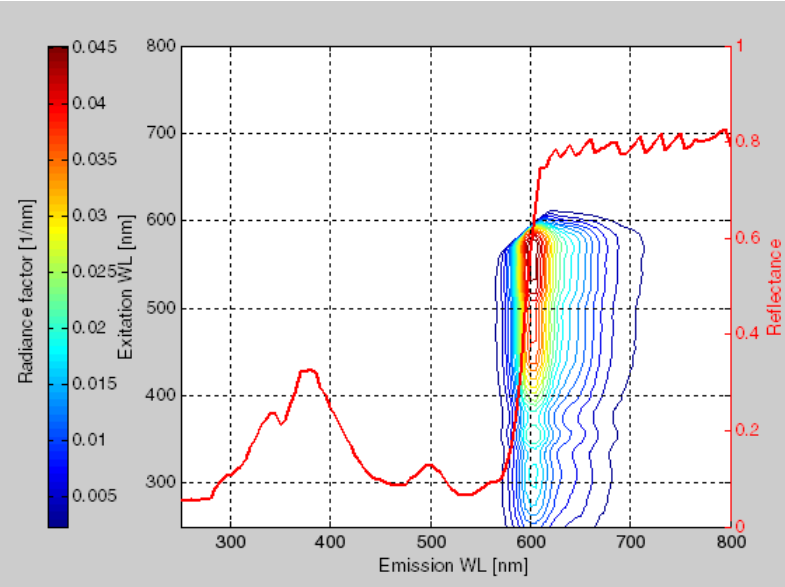

Figure 22: Fluorescent orange sample

Figure 21 shows that excitation and emission wavelength bands of fluorescent orange sample are in the range of approximately 250-610 nm and 580-690 nm respectively.

Obtained total radiance factor of the fluorescent sample can be simulated under any given light source which radiates in the range of bispetral measurements. Fluorescent green sample simulations under CIE standard light sources and different color LEDs are shown in Figures 23 and Figure 24 respectively.

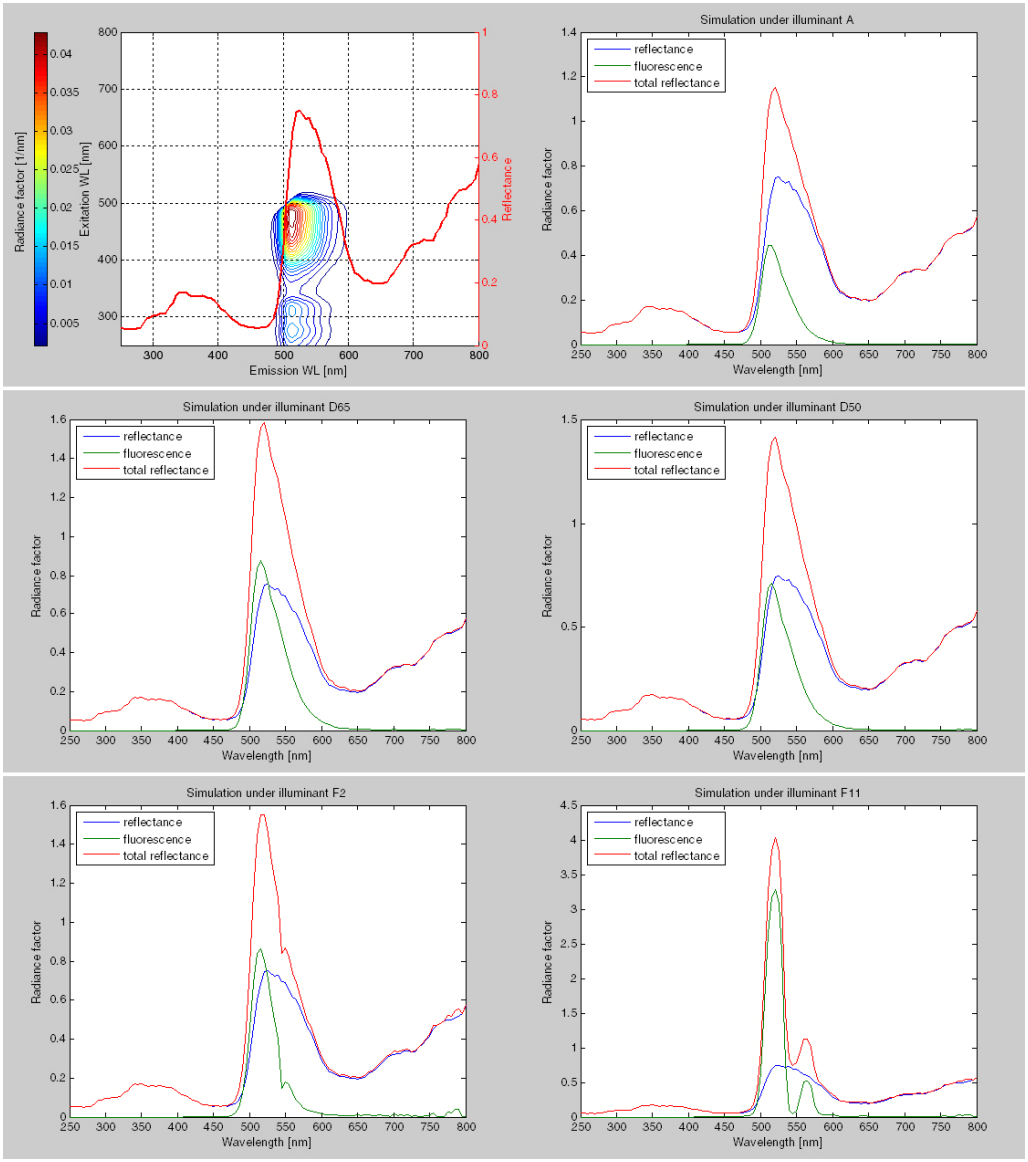

Figure 23: Fluorescent green sample simulations under CIE light sources

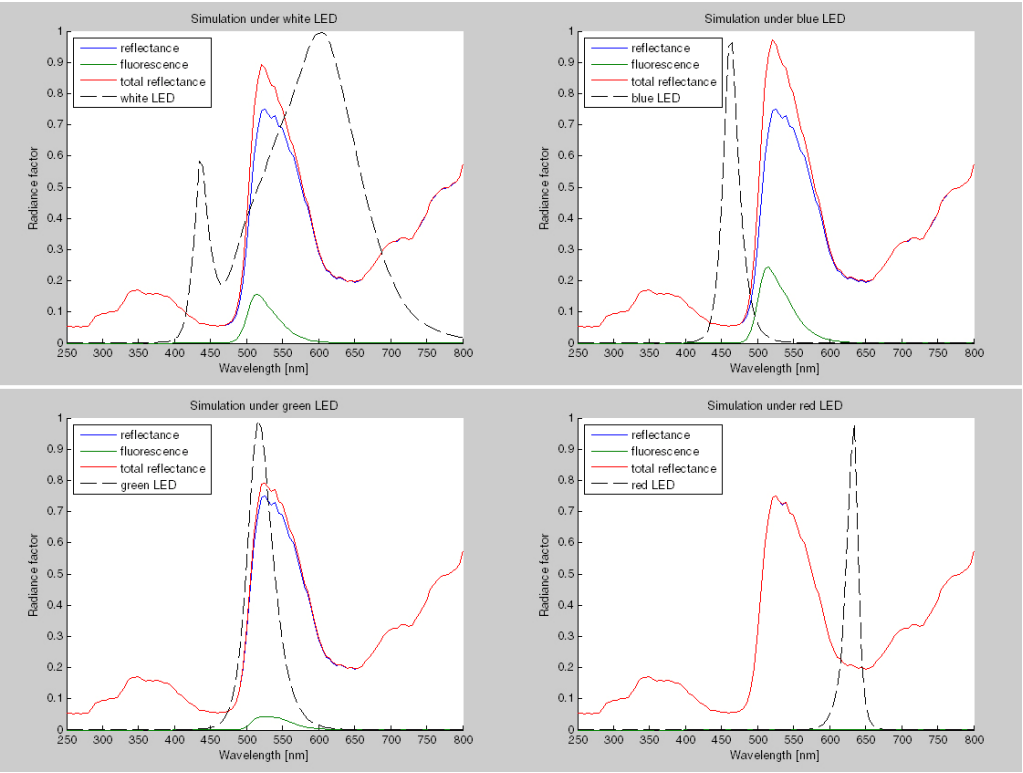

Figure 24: Fluorescent green sample simulated under different color LEDs

Figure 23, up left shows that fluorescence in green sample is expected to produce emission in the range of 490-590 nm with the maximum at approximately 510 nm. All light sources radiating from 250-510 nm would induce fluorescence. By irradiating the samples with CIE standard light sources we were not able to effectively observe fluorescent contribution to the color of the sample because the light sources cover mostly the whole visible spectrum, however by irradiating the samples with narrow band different color LEDs we could efficiently observe fluorescent component. As stated before light sources radiating from 250-510 nm would induce fluorescence in green sample. When excited with light sources with much energy in region from 400-500 nm green sample should exhibit the strongest fluorescence, however when excited with light sources with spectrum anywhere from 520-780 nm green sample should not exhibit fluorescence at all, which is demonstrated in Figure 24 down right.

After obtaining the Donaldson matrix, and fluorescence radiance factor, radiance factor and total radiance we were able to calculate RGB values of the sample. Table 1 shows the RGB values of each of these three radiance factors of green, yellow and orange fluorescent sample simulated under CIE Light sources.

D65

Fluorescent yellow	R	G	B
simulated reflected β_R	0.9580	0.8939	0.0000
fluorescent β_L	0.0000	0.8777	0.0000
total β_T	0.8977	1.0000	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.4685	0.8297	0.1405
fluorescent β_L	0.0000	0.7525	0.0000
total β_T	0.4381	1.0000	0.0532

Fluorescent orange	R	G	B
simulated reflected β_R	0.8793	0.3026	0.3481
fluorescent β_L	1.0000	0.3140	0.0000
total β_T	1.0000	0.4300	0.2772

D50

Fluorescent yellow	R	G	B
simulated reflected β_R	1.0000	0.8819	0.0000
fluorescent β_L	0.0000	0.7606	0.0000
total β_T	0.9836	1.0000	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.5279	0.8179	0.0000
fluorescent β_L	0.0000	0.6583	0.0000
total β_T	0.3439	1.0000	0.0000

Fluorescent orange	R	G	B
simulated reflected β_R	0.9326	0.2909	0.2863
fluorescent β_L	1.0000	0.2828	0.0000
total β_T	1.0000	0.4014	0.2033

A

Fluorescent yellow	R	G	B
simulated reflected β_R	1.0000	0.8212	0.0000
fluorescent β_L	0.0000	0.5207	0.0000
total β_T	1.0000	0.9425	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.6979	0.7591	0.0000
fluorescent β_L	0.0000	0.4522	0.0000
total β_T	0.6522	0.8593	0.0000

Fluorescent orange	R	G	B
simulated reflected β_R	1.0000	0.2252	0.0939
fluorescent β_L	0.9699	0.2178	0.0000
total β_T	1.0000	0.3142	0.0000

F2

Fluorescent yellow	R	G	B
simulated reflected β_R	1.0000	0.8912	0.0000
fluorescent β_L	0.0000	0.6241	0.0000
total β_T	1.0000	1.0000	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.6517	0.7977	0.0000
fluorescent β_L	0.0000	0.5429	0.0000
total β_T	0.5761	0.9337	0.0000

Fluorescent orange	R	G	B
simulated reflected β_R	0.8703	0.3313	0.2531
fluorescent β_L	1.0000	0.2487	0.0000
total β_T	1.0000	0.4101	0.1696

F11

Fluorescent yellow	R	G	B
simulated reflected β_R	1.0000	0.8862	0.0000
fluorescent β_L	0.0000	0.5391	0.0000
total β_T	1.0000	1.0000	0.0000
Fluorescent green	**R**	**G**	**B**
simulated reflected β_R	0.5785	0.8185	0.0000
fluorescent β_L	0.0000	0.4703	0.0000
total β_T	0.5255	0.9178	0.0000
Fluorescent orange	**R**	**G**	**B**
simulated reflected β_R	1.0000	0.2696	0.2371
fluorescent β_L	0.9823	0.2337	0.0000
total β_T	1.0000	0.3553	0.1572

Table 2: RGB values of simulated reflected, fluorescent, and total reflectance of fluorescent yellow, green, and orange sample simulated under CIE light sources

Analysis of simulated results and RGB values reveals that contribution of a fluorescent component to the color of the sample is altered by illumination, thus the total reflectance and RGB values are affected. If we closely observe RGB values of fluorescent component of green sample simulated under CIE light sources (see Table 3) we can see that red and blue values are always zero, whereas green component value is always changing since the intensity of the fluorescence varies proportionally to the light sources. This behavior is expected if we observe again the Donaldson matrix graph of the fluorescent green sample and the contour in the graph which shows the fluorescence radiance factor. The sample is expected to fluoresce in the region from 490 – 580 nm with highest intensity in 510 nm.

Fluorescent green sample

Total radiance factor	R	G	B
D65	0.4381	1.0000	0.0532
D50	0.3439	1.0000	0.0000
A	0.6522	0.8593	0.0000
F2	0.5761	0.9337	0.0000
F11	0.5255	0.9178	0.0000

Fluorescence radiance factor	R	G	B
D65	0.0000	0.7525	0.0000
D50	0.0000	0.6583	0.0000
A	0.0000	0.4522	0.0000
F2	0.0000	0.5429	0.0000
F11	0.0000	0.4703	0.0000

Table 3: RGB values of total radiance factor and fluorescent radiance factor of fluorescent green sample simulated under CIE light sources

As mentioned earlier by irradiating the samples with CIE standard light sources we couldn't effectively observe fluorescent contribution to the color of the sample because the light sources cover mostly the whole visible spectrum. This is recognized in Table 3 - if we observe RGB values of total radiance factor, we can see that except for the blue component which is the same under all light sources, red and green values vary under different light sources.

By irradiating the samples with narrow band different color LEDs we could efficiently observe fluorescent component. Table 4 shows the RGB values of simulated reflected radiance factor, fluorescent, and total radiance factor of green, yellow and orange fluorescent sample simulated under different color LEDs.

White LED

Fluorescent yellow	R	G	B
simulated reflected β_R	0.9579	0.8938	0.0000
fluorescent β_L	0.0000	0.3735	0.0000
total β_T	0.9434	0.9501	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.4285	0.8296	0.1405
fluorescent β_L	0.0000	0.3296	0.0000
total β_T	0.4205	0.8777	0.1328

Fluorescent orange	R	G	B
simulated reflected β_R	0.8793	0.3026	0.3481
fluorescent β_L	0.6089	0.1245	0.0000
total β_T	1.0000	0.3296	0.3336

Blue LED

Fluorescent yellow	R	G	B
simulated reflected β_R	0.9579	0.8938	0.0000
fluorescent β_L	0.0000	0.4629	0.0000
total β_T	0.9391	0.9807	0.0000

Fluorescent green	R	G	B
simulated reflected β_R	0.4385	0.8296	0.1405
fluorescent β_L	0.0000	0.4159	0.0000
total β_T	0.3938	0.9061	0.1234

Fluorescent orange	R	G	B
simulated reflected β_R	0.8793	0.3026	0.3481
fluorescent β_L	0.4689	0.1104	0.0000
total β_T	0.9701	0.3250	0.3392

Green LED

Fluorescent yellow	R	G	B
simulated reflected β_R	0.9579	0.8938	0.0000
fluorescent β_L	0.0497	0.2351	0.0000
total β_T	0.9597	0.9165	0.0000
Fluorescent green	**R**	**G**	**B**
simulated reflected β_R	0.4385	0.8296	0.1405
fluorescent β_L	0.0000	0.1974	0.0000
total β_T	0.4311	0.8475	0.1296
Fluorescent orange	**R**	**G**	**B**
simulated reflected β_R	0.8793	0.3026	0.3481
fluorescent β_L	0.6079	0.1448	0.0000
total β_T	1.0000	0.3371	0.3327

Red LED

Fluorescent yellow	R	G	B
simulated reflected β_R	0.9579	0.8938	0.0000
fluorescent β_L	0.0000	0.0039	0.0000
total β_T	0.9579	0.8940	0.0000
Fluorescent green	**R**	**G**	**B**
simulated reflected β_R	0.4385	0.8096	0.1405
fluorescent β_L	0.0000	0.0025	0.0000
total β_T	0.4384	0.8097	0.1405
Fluorescent orange	**R**	**G**	**B**
simulated reflected β_R	0.8793	0.3026	0.3481
fluorescent β_L	0.0287	0.0000	0.0000
total β_T	0.8804	0.3026	0.3480

Table 4: RGB values of simulated reflected, fluorescent and total radiance factor of green, yellow and orange sample simulated under LEDs

Fluorescent yellow sample

Total radiance factor	R	G	B
White LED	0.9434	0.9501	0.0000
Blue LED	0.9391	0.9807	0.0000
Green LED	0.9597	0.9165	0.0000
Red LED	0.9579	0.8940	0.0000

Fluorescence radiance factor	R	G	B
White LED	0.0000	0.3735	0.0000
Blue LED	0.0000	0.4629	0.0000
Green LED	0.0497	0.2351	0.0000
Red LED	0.0000	0.0039	0.0000

Table 5: RGB values of total radiance factor and fluorescent radiance factor of fluorescent yellow sample simulated under LEDs

Comparison of the RGB values of a fluorescent radiance factor shows that fluorescent yellow sample exhibits the fluorescence in green part of the visible spectrum. This is rather expected since this sample fluoresce in the region from 490-600 nm with peak in approximately 505 nm.

The analysis of the RGB values of total radiance factor of fluorescent yellow sample simulated under different LEDs reveals that there is considerable difference in values of green component (see Table 5). The blue value doesn't change while difference in red value is insignificant. When sample is simulated under blue LED the green component value is 0.9807 while it equals to 0.8940 when simulated under red LED. There is a clear difference between this two values which suggests that this sample is fluorescent.

5.2 LCTF

LCTF camera was used to conduct the measurements of fluorescent samples in the light boot. In addition, non-fluorescent Macbeth color checker was used as a reference in the measurements. The idea in this approach is to recognize if a sample is fluorescent by comparing the RGB values of fluorescent samples to similar color non-fluorescent samples. The RGB values are derived from the images taken both under a desired light source and under the combination of that same light source with UV light.

The results of the measurements of fluorescent yellow, green, and orange post-it paper are shown in Table 6.

Fluorescent yellow sample	R	G	B
D65	0.8220	0.9589	0.1121
D65 +UV	0.8202	0.9602	0.1356
A	1.0000	0.7886	0.0000
A +UV	1.0000	0.8637	0.0001
Horizon	1.0000	0.7130	0.0000
Horizon +UV	1.0000	0.8637	0.0001
Cool white	0.9131	0.8450	0.0000
Cool white +UV	0.9052	0.8829	0.0001
TL84	0.9874	0.7750	0.0000
TL84 +UV	0.9798	0.7945	0.0000

Fluorescent green sample	R	G	B
D65	0.2747	0.8981	0.1570
D65 +UV	0.2655	0.9024	0.1611
A	0.5763	0.7318	0.0000
A +UV	0.5674	0.7804	0.0003
Horizon	0.6516	0.6612	0.0000
Horizon +UV	0.6415	0.7354	0.0025
Cool white	0.5310	0.7538	0.0000
Cool white +UV	0.5073	0.7791	0.0003
TL84	0.5366	0.7065	0.0002
TL84 +UV	0.5375	0.7192	0.0003

Fluorescent orange sample	R	G	B
D65	1.0000	0.3877	0.3104
D65 +UV	1.0000	0.3878	0.3154
A	1.0000	0.3051	0.1059
A +UV	1.0000	0.3300	0.1169
Horizon	1.0000	0.2706	0.0820
Horizon +UV	1.0000	0.3506	0.1059
Cool white	1.0000	0.3879	0.0838
Cool white +UV	1.0000	0.3995	0.1304
TL84	1.0000	0.3417	0.0588
TL84 +UV	1.0000	0.3561	0.0889

Table 6: RGB values of fluorescent yellow, green, and orange sample under different light sources

Analysis of the results in Table 6 reveals that measurements under A light source, and A plus UV light show significant difference in RGB values. The same applies to horizon, and combination of horizon and UV light. Whereas other light sources were not suitable in detecting this difference.

Table 7 shows RGB values of fluorescent yellow sample and non-fluorescent yellow patch of Macbeth chart of measurements taken under A and A plus UV, and horizon and horizon plus UV light.

Fluorescent yellow sample	R	G	B
A	1.0000	0.7886	0.0000
A +UV	1.0000	0.8637	0.0001
Macbeth yellow patch	**R**	**G**	**B**
A	0.9777	0.6126	0.0001
A +UV	1.9762	0.6111	0.0002

Fluorescent yellow sample	R	G	B
Horizon	1.0000	0.7130	0.0000
Horizon +UV	1.0000	0.8073	0.0001
Macbeth yellow patch	**R**	**G**	**B**
Horizon	1.9998	0.5824	0.0000
Horizon +UV	1.0000	0.5822	0.0000

Table 7: RGB values of fluorescent yellow and similar yellow color Macbeth patch

Comparison of the RGB values of a fluorescent yellow sample under A light source and under the combination of A and UV light sources reveals the considerable difference in green component while red and blue components show no difference. RGB values under the horizon light and combination of horizon and UV light change in the same manner as in previous case.

Now, if we observe RGB values of Macbeth yellow patch under the same measurement conditions as for fluorescent yellow sample, we can see that there is no significant difference in values of the measurements taken under A light source and under combination of A and UV light, nor under horizon and horizon plus UV light. This finding suggest that sample whose RGB values are altering is fluorescent.

6 Conclusion

This thesis work introduces the methods for estimating the part of fluorescent radiation in image, and recognizing the fluorescence from RGB values of an image. Computational methods have been used to estimate fluorescence from bispectrometer measurements, and to estimate influence of fluorescence from a RGB image.

Basic mechanism of a fluorescence phenomenon was described, and some of the materials that exhibit fluorescence and their applications were presented. Several fluorescence measurement methods have been studied and evaluated. One-monochromator and two-monochromator methods are the main methods for measuring the spectrum of fluorescent samples. Two-monochromator method is considered as a reference method. Some of the one-monochromator methods which include filters, such as serial filter method, with suitable calibration and automation can be equivalent to two-monochromator method. When taking the measurements with bispectrometer we should consider calibration and device specific error corrections.

Spectral variables and colorimetric values for the bispectrometer measurements were derived. Donaldson matrix is explained in detail and illuminants used in the measurements are briefly overviewed. By analyzing the simulated results and RGB values we found that contribution of the fluorescent component to the color of the sample is altered by illumination, thus the total reflectance and RGB values are affected.

Two approaches for recognizing the fluorescence were presented. Bispectrometer approach involves comparison of RGB values derived from the same image taken under different light sources, while LCTF method relies on RGB comparison of two images taken under the same light source.

The methods proposed in this study are foundation for further research. By analyzing the RGB values of an image we showed that it is possible to recognize weather the sample is fluorescent or not. The development of the algorithm which would be able to estimate the fluorescence from a RGB image is considered for future work. It is necessary to increase the number of samples and to introduce more light sources. Additional measurements in a more controlled environment are required.

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